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Improved elongation factor-1 alpha-based vectors for stable high-level expression of heterologous proteins in Chinese hamster ovary cells

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Abstract

Background: Establishing highly productive clonal cell lines with constant productivity over 2-3 months of continuous culture remains a tedious task requiring the screening of tens of thousands of clonal colonies. In addition, long-term cultivation of many candidate lines derived in the absence of drug selection pressure is necessary. Expression vectors based on the elongation factor-1 alpha (EEF1A) gene and the dihydrofolate reductase (DHFR) selection marker (with separate promoters) can be used to obtain highly productive populations of stably transfected cells in the selection medium, but they have not been tested for their ability to support target gene amplification under gradually increasing methotrexate pressure.Results: We have modified EEF1A-based vectors by linking the DHFR selection marker to the target gene in the bicistronic RNA, shortening the overall plasmid size, and adding an Epstein-Barr virus terminal repeat fragment (EBVTR) element. Presence of the EBVTR element increased the rate of stable transfection by the plasmid by 24 times that of the EBVTR-minus control and improved the rate of methotrexate-driven gene amplification. The mean expression level of the enhanced green fluorescent protein (eGFP) used herein as a model protein, increased up to eight-fold using a single round of amplification in the case of adherent colonies formation and up to 4.5-fold in the case of suspension polyclonal cultures. Several eGFP-expressing cell populations produced using vectors with antibiotic resistance markers instead of the DHFR marker were compared with each other. Stable transfection of Chinese hamster ovary (CHO) DG44 cells by the p1.2-Hygro-eGFP plasmid (containing a hygromycin resistance marker) generated highest eGFP expression levels of up to 8.9% of the total cytoplasmic protein, with less than 5% of the cell population being eGFP-negative.Conclusions: The p1.1 vector was very effective for stable transfection of CHO cells and capable of rapid MTX-driven target gene amplification, while p1.2-Hygro achieved similar eGFP expression levels as p1.1. The set of vectors we have developed should speed-up the process of generating highly productive clonal cell lines while substantially decreasing the associated experimental effort.

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Keywords

CHO cells, High level expression, Molecular cloning, Stable cell line generation